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**WO 02/31183 A1**

(54) Title: NANOSCALE SENSOR

(57) Abstract: In the present invention, organic, inorganic, or other molecules or objects that may participate in an interaction event, are physically linked to nanoscale objects, typified, but not limited to, carbon nanotubes, silicon nanotubes, nanobars, and biological structural elements, such as microtubules and actin filaments, to create a signal transduction system that reports events on the micron scale or smaller. A molecular interaction even is monitored utilizing an amp meter or utilizing a scanning tunneling microscope so that when the molecules or objects participate in a molecule interaction event, or some other interaction event, the movement or change in the physical properties of the nanotube is monitored to report the occurrence of the interaction event.

**NANOSCALE SENSOR****PRIORITY**

This application claims benefit from prior Provisional Application Serial No. 60/238,518, filed October 10, 2000 and U.S. Patent Application Serial No. \_\_\_\_\_, filed on October 9, 2001. The invention disclosure of Provisional Application Serial No. 60/238,518 and U.S. Non-Provisional Application Serial No. \_\_\_\_\_ are hereby incorporated by reference.

**FIELD**

The present invention is an apparatus and method of detecting nanoscopic events. In particular, the present invention is a method of monitoring molecular interaction events by monitoring the effect the interaction event has on nanoscale objects to which one or more of the molecules or objects involved in the event are bound.

**BACKGROUND**

Detecting events that occur on the nanometer scale and reporting these events in the macroscopic world are of great utility. Such events include molecular interaction events between biomolecules, antibodies and antigens, enzymes, and other chemical and inorganic molecular events. One prior art method for detecting events on the nanometer scale is through the use of changes in averaged phenomena. Such methods include population methods. Population methods, however, require the use of large numbers of molecules and only report the averaged characteristics of the entire population. Recently, further methods have been developed that allow the investigation of small populations of molecules, or even

single molecules. Scanning probe microscopy and high resolution optical methods are among these newly developed methods.

In the scanning probe method, a probe that is on the micron dimensional scale is used to interrogate topographic or other features of molecules on a solid support. In one variation, chemical, biomolecular or modified microparticles can be attached to the end of the probe and extremely fine force measurements can be taken as the microparticle is scanned across a surface or a sample on the surface. These force measurements can tell when the microparticle attached to the end of the probe has an interaction with the surface or an object deposited on the surface.

One problem with the above methods of study is that the "reporter" systems involve an object that is on the micron spatial scale or larger. In contrast, the molecules of interest are often on the nanometer scale or smaller; the activities of the molecules of interest may also occur on the nanometer scale. Detection of nanometer scale molecular interaction events is difficult even utilizing advanced AFM probe technology. Compounding the problem is the fact that the probe to which the molecule is tethered is much larger and heavier than the molecule tethered thereon, or the molecule of interest. Because of the large size discrepancies between the two molecules and the probe, low or reduced sensitivity to single molecular interaction events may result. A need therefore exists for improved apparatuses and methods of detecting molecular interaction events on the micron and nanometer scale.

Accordingly, there is a need for an apparatus and method for detecting molecular interaction events that occur on the nanometer scale. There is a further need for directly detecting these molecular interaction events without resorting to a population average. Finally, there is a need for an apparatus and method that can act as a reporter for singular biomolecular interaction events.

## SUMMARY

The present invention describes an apparatus and method for using nanotubes and other nanoscale objects as part of a reporter system that enables the detection of the activities of molecules and other objects that are on the micron scale or smaller. This new apparatus and method may have improved sensitivity and responsiveness to molecules and molecular activities that occur on the nanometer scale.

In the present invention, organic, inorganic, or other objects that may participate in a molecular interaction event are physically linked to nanoscale objects, typified, but not limited to, carbon nanotubes, silicon nanotubes, metal nanobars, and biological structural elements, such as microtubules and actin filaments, to create a signal transduction system that reports molecular interaction events on the micron scale or smaller.

In one embodiment, a first nanoscale object is formed and operably positioned relative to a second nanoscale object which in turn has a molecule tethered at one end. The first nanoscale object is linked at each end thereof to individual micron sized conductive pads. An electric current is then passed through the first nanoscale object and monitored. When the molecule bound to the second nanoscale object participates in a molecular interaction event, the physical properties of the second nanoscale object change in such a manner that the electrical conductivity of the first nanoscale object is affected. In the simplest case, the second nanoscale object will physically move because of the small size of the nanoscale object relative to the molecule bound thereon, thus affecting the current flow or resistance of the first nanoscale object. This current flow or resistance can then be detected to report the molecular interaction event. As may be appreciated, other physical properties of the nanoscale object interactions may also be measured.

In a second embodiment of the present invention, a first nanoscale object is formed on a surface with a molecule tethered at one end and a conductive pad tethered at the other. A

scanning tunneling microscope is utilized to monitor the position and electronic characteristics of the nanoscale object. When a molecular interaction event occurs, the position or electronic nature of the nanoscale object changes. The change in position or electronic nature of the nanotube is detected and reported by the scanning tunneling microscope.

The present invention further comprises a nanoscale sensor comprising a first nanotube formed on a surface, a monitoring apparatus operably attached to the first nanotube, a second nanotube formed on the surface and in operable relationship with the first nanotube, and at least one molecule tethered to a first end of the second nanotube wherein when a molecular interaction event occurs involving the tethered molecule, the molecular interaction event is detected by the monitoring apparatus.

Another embodiment of the present invention comprises a method of detecting molecular interaction events including operably coupling a first nanotube to a second nanotube wherein the first nanotube is operably coupled at a first end to a first conductive pad and operably connected at a second end to a second conductive pad and wherein the second nanotube is operably coupled at a first end to a third conductive pad and at a second end to a molecule, applying an electrical current that runs through the first and second nanotube, and monitoring the current flow through the first and second nanotubes with an amp meter wherein when the biomolecule undergoes a molecular interaction event the change in conductivity of the first and second nanotube is detected by the amp meter.

Yet another method of detecting molecular interaction events comprises tethering a molecule to a first end of a nanoscale object, scanning the nanoscale object with a scanning tunneling microscope, and detecting movement of the nanoscale object when the molecule undergoes a molecular interaction event.

Also included herein is an apparatus for detecting a molecular interaction event comprising a first nanotube operably attached to a surface, a second nanotube operably attached to the surface and operably connected to the first nanotube, a molecule tethered to a first end of the second nanotube wherein when the molecule participates in a molecular interaction event which results in a measurable change in the physical properties of the first and second nanotube.

### **SUMMARY OF THE DRAWINGS**

Figure 1 represents a schematic of the nanotube circuit of one embodiment of the present invention.

### **DETAILED DESCRIPTION**

As utilized in the present invention, nanoscale objects can be monitored in such a manner wherein changes in the physical position or other properties of the object can be recognized as an indication of a nanoscale event. Carbon nanotubes, for example, conduct electrons in such a manner that they can act like wires, switches, and diodes. Electrical resistance is therefore one property that can be monitored to indicate the occurrence of a molecular interaction event. Other nanoscale objects may also be utilized in the present invention. Such nanoscale objects include silicon nanotubes, biological structural elements, such as microtubules and actin filaments, as well as nanobars and nanowires. The present invention will utilize carbon nanotubes as the preferred nanoscale object. As may be appreciated, the well defined metallic properties, and the ability to create nanobars with defined physical and chemical attributes, may provide an alternative apparatus for the construction of the nanosensors described herein. Furthermore, a system of this type may be easier to construct in bulk. Substitution of other nanoscale objects, therefore, such as those

listed above, may be accomplished by those skilled in the art without changing the nature and scope of the present invention.

The synthesis of  $C_{60}$  fullerene structures in the form of needle-like single or multilayer tubes having nanometer dimensions is known in the art. These extended fullerene tube structures are also known as carbon nanotubes, or thin walled carbon nanotubes. Structurally, carbon nanotubes are a member of a class of molecular carbons known as fullerenes. The atomic arrangement of the hexagonal carbon ring relative to the nanotube axis varies from tube to tube and is usually helical. The nanotubes are composed of a number of concentric cylindrical sheets (ranging from one to approximately 50) of hexagonally-arranged carbon atoms. The tubes typically have a diameter of a few to a few tens of nanometers and a length of up to a few micrometers. Such nanotubes are well known to those in the art.

The synthesis of these nanotubes in experimental quantities using a standard carbon arc method is known. Production of nanotubes typically consists of placing two water-cooled carbon electrodes of amorphous carbon or graphite rods approximately one millimeter apart within a vacuum chamber, evacuating the chamber to a pressure of approximately  $10^{-7}$  torr, backfilling the chamber with an inert gas such as helium, nitrogen, argon or hydrogen to pressures ranging from approximately 50 to 500 torr and then striking a high current electrical arc between the electrodes while continually adjusting them to maintain the one millimeter electrode gap. The result is a growth of carbon nanotubes and other small carbon particles on the negative electrode. The quantity of nanotubes produced in the electrode deposits depends on how long optimum growth conditions can be maintained.

Probes built this and other ways can be utilized for a number of experiments. See Wong, S.S., E. Joselevich, A.T. Woolley, C.L. Cheung, and C.M. Lieber, *Covalently functionalized nanotubes as nanometre-sized probes in chemistry and biology*, Nature, 1998,

p. 52-5; Wong, S.S., A.T. Woolley, E. Joselevich, C.L. Cheung, and C.M. Lieber, *Covalently-functionalized single-walled carbon nanotube probe tips for chemical force microscopy*, Journal of the American Chemical Society, 1998, p. 8557-8558; Wong, S.S., A.T. Woolley, E. Joselevich, and C.M. Lieber, *Functionalization of carbon nanotube AFM probes using tip-activated gases*, Chemical Physics Letters, 306, p. 219; Dai, H., E.W. Wong, and C.M. Lieber, *Probing electrical transport in nanomaterials: conductivity of individual carbon nanotubes*, Science, 1996, 272: p. 523-526; Shoushan Fan, Michael Chapline, Nathan Franklin, Thomas Tombler, A. Cassell and Hongjie Dai, *Self-Oriented Regular Arrays of Carbon Nanotubes and Their Functional Devices*, Science, 283, 512 (1999); Hyonsok T. Soh, Alberto Morpurgo, Jing Kong, Charles Marcus, Calvin Quate and Hongjie Dai, *Integrated Nanotube Circuits: Controlled Growth and Ohmic Contacts to Single-Walled Carbon Nanotubes*, Appl. Phys. Lett., 75, 6951, (1999); Hongjie Dai, Jing Kong, Chongwu Zhou, Nathan Franklin, Thomas Tombler, Alan Cassell, Shoushan Fan and Michael Chapline, *Controlled Chemical Routes to Nanotube Architectures, Physics and Devices*, J. Phys. Chem B. 1999, 103 11246-11255 (1999); Martin, B.R., Nanobars, Advanced Materials, 11, p. 1021 (1999); Cui, Y. et al., *Nanowire Nanosensors for Highly Sensitive and Selective Detection of Biological and Chemical Species*, Science, 293, p. 1289 (2001); Martin, B.R. Nanobars, Advanced Materials, 11, 1021 (1999).

In such experimental setups, a DC voltage of about 18V is applied between two carbon electrodes in a chamber under about 500 torr of helium. A plasma forms between the closely-spaced electrodes. Carbon accumulates on the negative electrode and grows as the positive electrode is consumed. When the correct electrode spacing is maintained, the deposit grows into a cylindrical structure with an outer hard shell and an inner soft fibrous core. The gray outer shell is composed of carbon nanotubes and other carbon nanoparticles fused into a hard mass. The soft black inner core contains free nanotubes and nanoparticles



in the form of fibers where the fibers are aligned with the direction of current flow between the electrodes. Additional methods of forming nanotubes are known, such as those described in U.S. Patent 5,753,088 and 5,482,601.

The present invention apparatus and method describes an apparatus and method for utilizing carbon nanotubes to report events as macroscopic signals. The present invention apparatus and method will be described first; thereafter some specific examples will be described that utilize the method and apparatus as a nanoscale reporter.

One embodiment of the present invention apparatus includes a first carbon nanotube 10, a second carbon nanotube 12, and a surface 14. The first and second carbon nanotubes 10, 12 are operably connected and affixed to the surface 14. The first carbon nanotube 10 further comprises a conductive pad 16 operably attached to one end. The conductive pad 16 is also operably attached to the surface 10. The second carbon nanotube 12 may further comprise a conductive pad 18 attached to a first end and a molecule 20 tethered to a second end. The molecule 20 tethered to the second nanotube 12 is the molecule to be studied.

In the present embodiment, the conductive pads 16, 18 are formed of platinum grids and attached to the carbon nanotubes 10, 12. Other conductive pads, such as micron sized gold islands, may also be utilized.

The length and size of the nanotubes constructed may be determined by those skilled in the art depending on the nature of the molecular interaction event to be detected. Various methods allow reliable electrical connections to be made that are bridged by individual nanotubes. For example, methods of achieving semiconducting single walled carbon nanotubes which are then connected to two metal electrodes, such as the platinum electrode of the present embodiment, are described in *supra*, Nature, 292, (1998). Furthermore, carbon nanotubes have been shown to be grown in such a manner that they may integrated into electronic circuits. *See supra* J. Phys. Chem B. 1999, 103 11246-11255 (1999). The vapor

deposition creation of nanotubes utilizing substrates patterned with 1-5 micron wide catalytic islands can be utilized to grow nanotube chips in a desired location on the substrate. The position of the nanotubes can be confirmed utilizing atomic force microscopy or scanning tunneling microscopy. Utilizing various methods, for example, chemical vapor deposition may allow for single walled or multiwalled carbon nanotubes that are grown on a silicon surface that includes a silicon oxide substrate.

Next, a molecule 20 is tethered to the free end of the second nanotube 12 utilizing known techniques. The complexed end of the second nanotube 12 may have an inorganic or organic molecule 20 attached thereto depending on the molecular interaction event of interest. The modification of the tip in one embodiment is accomplished by coupling an amine to a pendant carboxyl group. In alternative embodiments, covalently modified nanotube tips, which have been previously described, may also be utilized. *See Supra*, Nature, 395, July 1998. In yet another alternative embodiment, the carboxyl group may be modified in such a manner as to provide even further complexes. As may be appreciated, the molecule 20 does not necessarily have to be tethered to the end of the second nanotube 12, but may also be tethered to a position along the entire length of the second nanotube 12.

The present embodiment may also further comprise a power source 22. The power source 22 is operably attached to the conductive pad 16 attached to the first nanotube 10 and the conductive pad 18 attached to the second nanotube 12. The power source 22 allows for a small voltage to be applied through the first and second carbon nanotubes 10, 12. The power source 22 may further comprise controls so that the amount of power supplied to the nanotubes 10, 12 can be closely monitored and controlled. These circuits formed by the nanotube bridges may also be characterized by measuring the electrical resistance or conductance.

The present embodiment may further comprise an amp meter 24. The amp meter 24 is operably attached to the first and second carbon nanotubes 10, 12 to monitor the current flow in the same. An amp meter 24 that can measure resistance on the nanoamp and picoamp scale is known to those skilled in the art.

Due to the electronic nature of nanotubes 10, 12, the shape of the nanotube structure may have various electronic properties that can be controlled by virtue of the chemical details of the nanotubes used. By virtue of the conductive pads 16, 18 and the power source 22, the intersection between the first and second nanotubes 10, 12 can be a conductive pad, a rectifying path, a switched path, or even a transistor. In the present embodiment, the intersection creates a conductive pad that is monitored by the user. The electronic signature of the nanotube contact is measured and used as a baseline prior to activation of the enzyme. When a molecular interaction event occurs involving the tethered molecule 20, the dynamic molecular characteristics are transduced by the nanotubes 10, 12 and amplified into macroscopic signals. An alteration in their physical orientation, for example, results in a change in the amount of current flowing and so may be measured by monitoring the resistance of the system.

When utilizing the present invention as a molecular event detector, the molecule 20 attached to the second nanotube 12 is exposed to a target sample. The target sample may contain a material that is potentially reactive with the molecule 20 tethered to the end of the second nanotube 12. By being potentially reactive, the present invention contemplates that if the molecule is present, it will react in a detectable way with the tethered molecule. The reaction may be any molecular interaction event that results in some reportable change in the system. The present embodiment contemplates changes in the electrical conductivity and may be detected by the amp meter. As may be appreciated by those skilled in the art, the

teachings of the present invention method may also be applicable to a wide range of selected systems.

As previously mentioned, when the molecule 20 tethered to the end of the second nanotube 12 undergoes a molecular interaction event, the event causes a change in the physical relationship between the first nanotube 10 and the second nanotube 12. In the present embodiment, the change is reported by a change in the monitored voltage or resistance as measured through the platinum pads 16, 18 on the ends of the first and second nanotubes 10, 12. This change in resistance can be caused by a change in the physical relationship of the nanotubes 10, 12 resulting from the molecular interaction event, i.e. changing length of the path of current, thereby changing the resistance as detected by the amp meter 24. In other words, since each of the nanotubes 10, 12 are connected to a macroscopic circuit through the conductive pads 16, 18, measuring the changes in current flow through the two nanotubes 10, 12 is related to the molecular activity occurring.

Properties of the nanotubes such as, but not limited to, motion, thermal conductivity, resistance, compression, expansion, etc. may vary and be detected in alternative embodiments. The changes in the properties of the nanotubes alters the nature of the nanotube-nanotube contact, thereby reporting to the macroscopic world the nature of the enzyme activity. As may be appreciated by those skilled in the art, in alternative embodiments, various properties of the first and second nanotubes 10, 12 may be monitored to detect the occurrence of the molecular interaction event.

In one alternative embodiment of the present invention, only one nanotube may be utilized to report the molecular interaction event. The one nanotube is formed on the selected surface and a molecule is tethered thereto. A scanning tunneling microscope is operably positioned relative to the nanotube in order to monitor the position or electronic characteristics of the same. When a molecular interaction even occurs to the molecule

tethered to the end of the nanotube, the scanning tunneling microscope reports the change in position of the nanotube, thus reporting the occurrence of the interaction event.

In another alternative embodiment of the present invention, the present invention may include a first nanotube, a second nanotube, a first conductive pad, and a second conductive pad attached to the first nanotube and a second conductive pad connected to the second nanotube. The nanotubes are operably affixed relative to one another and a molecule is tethered to the end of the second nanotube. The embodiment also includes a power source and an amp meter connected between the pads. In this embodiment the electrical flow goes through the first pad attached to the first nanotube, crosses over to the second nanotube, and then goes through the second nanotube and to the second pad. As may be appreciated, the molecule tethered to the end of the second nanotube in this embodiment should not be affected by the small electrical flow that is placed on the system. As with the previous embodiments, a molecular interaction event occurring with the molecule affects the current flowing through the system and is reported by the amp meter.

The below embodiments are presented to teach the invention and are representative of various alternative applications of the invention. The below embodiments are not intended to limit the invention in any way, as it may be applied to chemical sensing, material interactions, and other physical or electronic interactions.

#### **Example 1: Detecting Airborne Particles**

Airborne molecules may affect the recipient's behavior and general physical state. Since airborne molecules can cause both behavioral and physiological effects in animals or humans, even in low quantities, it is of great interest to be able to detect airborne particles. Such airborne particles may include various toxins, chemicals, or other agents such as pheromones.

In one example, a receptor for a pheromone is attached to a first nanotube. The first nanotube is anchored at the other end and intersects a roughly perpendicular second nanotube that is connected at both ends to gold electrodes. A power source and an amp meter are further included and operably attached to the system. The steady state electronic signature of the system is established using a sensitive circuit established between the two gold electrodes. The pheromones are introduced into the atmosphere in the vicinity of the nanotube bound receptor. Upon binding to a pheromone, the receptor undergoes a structural change that affects the properties of the attached first nanotube. The molecular interaction event is reported by detecting a change in the circuit of the second nanotube. In alternative embodiments, this apparatus can be made sensitive to transient events by utilization of standard filtering techniques, such as implementation of a Kellman filter, known to practitioners of the art.

In alternative embodiments, other airborne pathogens may be detected utilizing the present invention. For example, the anthrax bacterium is a deadly pathogen that can be transferred as an airborne particle. Other materials that may be detected include mold spores and viral pathogens.

In these alternative embodiments, the molecule tethered to the nanotube may include aptamers. Aptamers are molecules that are designed to bind to specific biological or non-biological molecular surfaces and structures. Aptamers for a wide variety of interactions, and that react under a variety of conditions, can be created by methods known to those skilled in the art. When the aptamer interacts with the airborne pathogen, it undergoes a structural change that is transduced as in the example above and detected by the nanotube coupled circuitry.

In another alternative embodiment, the present invention may be utilized for photon detection. In this example a photoreceptor is bound to the end of a nanotube-based

nanosensor. The photon receptor could be based on any of a wide variety of photon sensors in biology, including the photoreceptors in the eye, the photoreceptor involved in the first step of photosynthesis, and the photoreceptors involved in harvesting light energy in cyanobacteria (also known as blue-green algae - a misnomer). Alternatively, the photoreceptor could be a natural or man-made, non-biological material such as a photoreactive mineral (e.g. silicon). In this example the interaction between photons of a defined energy level cause a structural change in the photodetector that is transduced through the nanoscale sensor. In another alternative embodiment, the present invention may be similarly utilized as a heat sensor. It is clear from these examples that sensors for a wide variety of interactions can be designed around the central concept presented. These include, but are not limited to: particle sensors, chemical sensors, and material sensors.

It should be noted that in the examples above the binding reaction occurs in air, and the detection is carried out based on sensing of a mechanically transduced structural change. In an alternative embodiment, the sensing event can be based on a change of the electronic state of the nanotube that is directly induced by the binding interaction. For example, a sensor is constructed such that a nanotube spans two gold electrodes, as described above. Tethered to the middle of the nanotube is a sensing entity such as the pheromone receptor described above. This tethering can be accomplished at defect regions known to occur in nanotubes. The electronic state of the sensor is established by measuring the circuit. Upon reaction with a pheromone ligand, this electronic state will change due to the addition of a new component to the system that merges its electronic characteristics with those of the preexisting system. This state change is then monitored by virtue of a change in the sensing circuitry.

Thus far, all examples have been described using systems operating in air at some level of humidity or in a vacuum. However, it is possible to carry out similar reactions in

solution as long as the sensor circuit can be protected and distinguished from the liquid environment. It is well known that scanning tunneling microscopes can be operated in solution by covering the bulk of the probe with an insulating material and leaving only the extreme apex open to the surrounding liquid environment. This same technique can be used to create nanoscale sensor systems with similar attributes. For example, a nanoscale sensor can be coated with an insulating material by ion beam sputtering the material over all but the sensing portion of the system. In this case, the system can be made to operate in solution, allowing the detection of biomolecular events in liquid systems. The following include examples that are of particular utility.

**Example 2: Detecting Enzyme Activity**

A first nanotube is formed as described above and linked to a biomolecule. The first carbon nanotube is treated with acid at one end to create dangling -COOH groups at the terminus end. The -COOH group are then coupled to an enzyme by condensation chemistry. In one embodiment of the present invention method, a carbodiimide reagent is used to catalyze the condensation of the free carboxyl group with a primary amine on the enzyme, thereby releasing a water molecule and forming a covalent tether between an enzyme and the nanotube.

The enzyme is next activated by addition of a substrate and any required cofactors. As the enzyme carries out its catalytic function, properties of the first and second nanotube vary in response to the catalytic function of the enzymes.

In one embodiment, the motion of enzyme linked during the molecular interaction event results in changes in the resistance of the circuit formed by the nanotubes. In one embodiment, the length of the circuit path changes when the molecular interaction event changes the relative position of the first and second nanotube. Changes in resistance are



measured by virtue of electrons drawn off through the second nanotube as it slides, moves, or reacts in any way with the first. In another alternative embodiment, other physical characteristics and relationships of the two nanotubes may be detected when the molecular interaction event occurs.

In one alternative embodiment, a structure such as an electron gate may be incorporated in the architecture of the crossed nanotubes, creating a transistor switch.

In the present example, the primary information derived is whether or not the enzyme is active and undergoes a molecular interaction event. This information can be of great utility if, for instance, the enzyme is only active in the presence or absence of some reagent. An example would be in sensing metal ions which catalyze a chemical reaction. Another example would be sensing a specific type of enzyme substrate whose presence or absence is a diagnostic for a specific condition. The present invention is useful for both of these characterizations.

In another alternative embodiment, when the enzyme is a DNA polymerase, the presence of a specific nucleic acid is detected by a change in the molecular shape or other characteristic. The nucleic acid is detected by virtue of the enzyme becoming active only when a complementary primer DNA molecule binds to the target DNA molecule, thereby forming the appropriate substrate upon which the polymerase acts.

In yet another embodiment of the present embodiment example, the binding of one protein to another is detected. When an antibody is built onto one of the nanotube sensors, the binding of the antibody to an antigen causes a change in the antibody structure. This change in structure is translated into an electronic signal in the same manner as previously described.

One advantage of the present embodiment of the present invention is that the nanotube sensor may be of a similar size to the enzyme. The similar size of the nanotube and

the enzyme means that the activity of the enzyme has a profound effect on the nanotube characteristics. This is in contrast to, for example, a conventional AFM probe, which has a mass many thousands of times larger than that of a single enzyme molecule attached to its apex. The difference in size results in low sensitivity.

### **Example 3: Detecting DNA Sequencing**

In this embodiment, a nanotube junction is created in which a DNA polymerase enzyme is tethered to one end of the second nanotube. In the present embodiment, a DNA substrate and a complementary primer are added to the system. When the substrate and primer bind together and bind to the tethered DNA polymerase, the enzyme is ready to act. Nucleotide triphosphate precursors, including appropriate buffers and catalyzers are added to begin the enzyme activity. As the enzyme adds nucleotides to the primer molecule, it creates signals effecting the electrical flow between the first and second nanotube in the nanotube junction that are the result of the energy expended by the enzyme, and that are detected by the amp meter. The generated signals can be mechanical, thermal, or some other form of energy. In the present embodiment, these signals correspond to the sequence of the DNA being synthesized by addition of nucleotides to the primer.

For a polymerase to become active, it requires a DNA substrate that contains both the target DNA strand and a primer strand. The primer strand presents a 3'-OH group to which the polymerase can begin to add nucleotides while copying the target strand. It is known that when a DNA polymerase acts to incorporate complementary nucleotides into a growing strand of DNA, the stereochemistry and energy requirements for each nucleotide type are slightly different. These differences constitute discrete indicators of which nucleotide, A, C, G, or T (U in RNA) are being incorporated. This information is transduced by the nanosensor described herein, resulting in immediate knowledge of the DNA sequence of the molecule being synthesized by the polymerase component of the nanosensor. The present

embodiment may be applicable for both DNA and RNA molecules. Thus, the present embodiment allows a user to analyze the signals generated to directly deduce the DNA or RNA sequence being generated. The utility and advantages of such an invention is obvious.

In an alternative embodiment, the above method may be carried out with a large ensemble of nanoscale sensors formed on the same surface. Each biosensor is approximately  $2\mu \times 2\mu$  in spatial dimension, so that millions of which can exist on a solid state substrate or  $1\text{ cm}^2$  "chip." Utilizing such a method and apparatus makes it possible to determine the sequence of DNA fragments simultaneously. In addition, since the reaction rates of DNA polymerases can be as high as 20-40 nucleotides per second or more, it translates into an extremely fast and efficient sequencing rate.

The information and examples described herein are for illustrative purposes and are not meant to exclude any derivations or alternative methods that are within the conceptual context of the invention. It is contemplated that various deviations can be made to this embodiment without deviating from the scope of the present invention. Accordingly, it is intended that the scope of the present invention be dictated by the appended claims rather than by the foregoing description of this embodiment.

All publications cited in this application are incorporated by reference in their entirety for all purposes.

**CLAIMS**

1. A sensor comprising:
  - a first nanoscale object formed on a surface;
  - a second nanoscale object formed on the surface and in operable relationship with the first nanoscale object;
  - a monitoring apparatus operably attached to the first nanoscale object and the second nanoscale object; and
  - at least one molecule tethered to a first end of the second nanoscale object wherein when a molecular interaction event occurs involving the tethered molecule, the molecular interaction event is detected by the monitoring apparatus.
2. The apparatus of claim 1 further comprising
  - a first conductive pad attached to a first end of the first nanoscale object; and
  - a second conductive pad attached to the second end of the second nanoscale object.
3. The apparatus of claim 1 further comprising a power source operably attached to the first and second conductive pad whereby the power source passes an electrical current through the first nanoscale object and the second nanoscale object and where the electrical current is detected by the monitoring apparatus.
4. The apparatus of claim 1 wherein the monitoring apparatus is an amp meter, the amp meter measuring the resistance of the electrical current through the first nanoscale object and the second nanoscale object and detecting the change in the same.
5. The apparatus of claim 1 wherein the surface is silicon.

6. The apparatus of claim 5 wherein the surface further comprises a layer of silicon dioxide.
7. The apparatus of claim 1 wherein the molecule tethered to the end of the second nanoscale object is a biomolecule.
8. The apparatus of claim 7 in which the biological molecule is an enzyme.
9. The apparatus of claim 8 in which the enzyme is a DNA polymerase.
10. The apparatus of claim 1 wherein the molecule tethered to the end of the second nanoscale object is an inorganic molecule.
11. A method of detecting molecular interaction events comprising  
operably coupling a first nanotube to a second nanotube wherein the first nanotube is operably coupled at a first end to a first conductive pad and a operably connected at a second end to a second conductive pad and wherein the second nanotube is operably coupled at a first end;  
applying a electrical current that runs through the first nanotube; and  
monitoring the current flow through the first nanotube with an amp meter wherein when the biomolecule undergoes a molecular interaction event it affects the conductivity of the first nanotube, the change in conductivity detected by the amp meter.
12. The method of claim 11 wherein the molecule is a biomolecule.

13. The method of claim 12 wherein operably coupling the biomolecule to the second nanotube further comprises performing a condensation reaction between a carboxyl group and a primary amine.
14. A method of detecting a molecular interaction event comprising:  
tethering a molecule to a first end of a nanoscale object;  
scanning the nanoscale object with a scanning tunneling microscope; and  
detecting movement of the nanoscale object when the molecule undergoes a molecular interaction event.
15. The method of claim 14 wherein the movement of the nanoscale object is detected through the change in the position of the nanoscale object relative to the probe of the scanning tunneling microscope.
16. The method of claim 14 wherein the molecule is a biomolecule.
17. The method of claim 14 wherein the molecule is an enzyme.
18. An apparatus for detecting a molecular interaction event comprising:  
a first nanotube operably attached to a surface;  
a second nanotube operably attached to the surface and operably connected to the first nanotube;  
a molecule tethered to a first end of the second nanotube wherein when the molecule participates in a molecular interaction event which results in a measurable change in the physical properties of the first and second nanotube.

19. The apparatus of claim 18 further comprising:

a monitoring device operably positioned relative to the first and second nanotube wherein when the molecular interaction even occurs the change in physical properties of the first nanotube and the second nanotube is detected by the monitoring device.

20. The apparatus of claim 18 wherein the monitoring device is an amp meter.

21. The apparatus of claim 19 wherein the monitoring device is a scanning tunneling microscope probe.

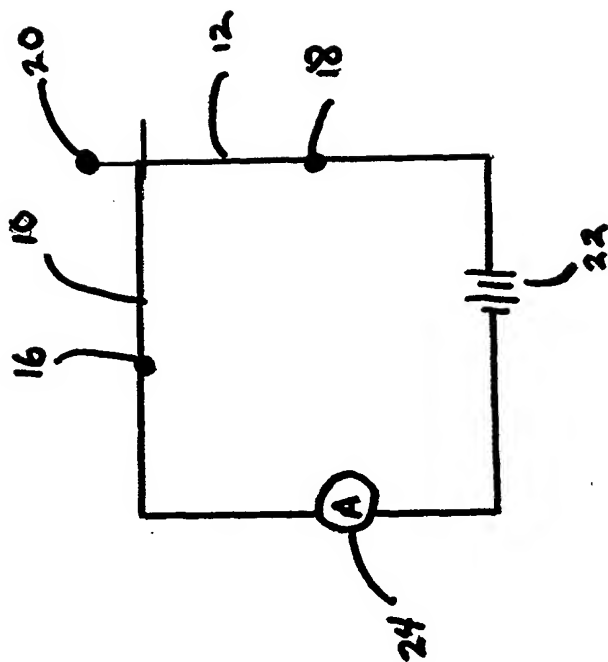


Figure 1



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/31615

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/00; C12M 1/34; G01N 27/26

US CL : 435/4, 287.1, 287.2, 817; 204/403

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 287.1, 287.2, 817; 204/403

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST

search terms: sensor, nanoscale, nanotubes, scanning tunneling microscope, enzyme

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 6,123,819 A (PEETERS) 26 September 2000, see entire document, especially column 5, lines 41-55, paragraph bridging columns 6 and 7, column 7, lines 38-60, and column 8, lines 30-37.	1, 5-7 ----- 2-4, 8-21
Y	US 5,866,434 A (MASSEY et al) 02 February 1999, see entire document.	2-4, 8-21
Y	US 5,346,683 A (GREEN et al) 13 September 1994, see entire document.	11-13, 18-21
Y	US 5,763,768 A (HENDERSON et al) 09 June 1998, see entire document.	1-21
Y	US 5,620,854 A (HOLZRICHTER et al) 15 April 1997, see entire document.	14-17, 21

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	"T" Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"G" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

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